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(54) Title: CRYSTALLINE OXATHIOLANE DERIVATIVES

(57) Abstract

(-)*cis*-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidine-2-one in crystalline form, in particular as needle-shaped or bipyramidyl crystals, pharmaceutical formulations thereof, methods for their preparation and their use in medicine.

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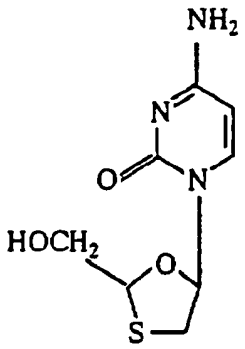
CRYSTALLINE OXATHIOLANE DERIVATIVES

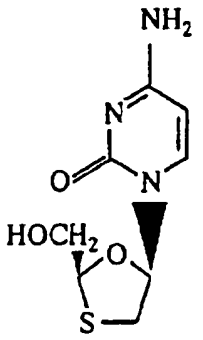
The present invention relates to nucleoside analogues and their use in medicine. More specifically the invention is concerned with 1,3-oxathiolane nucleoside analogues, particular physical form thereof, pharmaceutical formulations thereof and the use thereof in the treatment of viral infections.

The compound of formula (I)

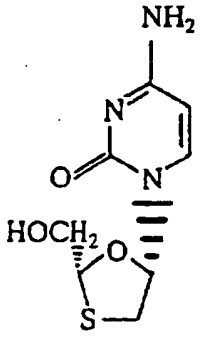


(I)

also known as BCH-189 or NGPB-21 has been described as having antiviral activity in particular against the human immunodeficiency viruses (HIV's), the causative agents of AIDS (5th Anti-Aids Conference, Montreal, Canada 5th-9th June 1989: Abstracts T.C.O.1 and M.C.P. 63; European Patent Application Publication No. 0382562). The compound of formula (I) is a racemic mixture of the two enantiomers of formulae (I-1) and (I-2):-



(I-2)



(I-1)

and was described and tested in the form of its racemate. The only compound currently approved for the treatment of conditions caused by HIV is 3'-azido-3'-deoxythymidine (AZT, zidovudine, BW 509U). However, this compound has a

significant side-effect liability and thus either cannot be employed or, once employed, may have to be withdrawn in a significant number of patients. There is in consequence a continuing need to provide compounds which are effective against HIV but with a concomitant significantly better therapeutic index.

Although the enantiomers of the compound of formula (I) are equipotent against HIV the(-)-enantiomer has considerably lower cytotoxicity than the other enantiomer and is thus the preferred compound as an antiviral agent.

The (-)-enantiomer has the chemical name (-)cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)- pyrimidin-2-one. It has the absolute stereochemistry of the compound of formula (I-1) which has the name (2R,cis))-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. The compound is now known as 3TC.

Preferably 3TC will be substantially free of the corresponding (+)-enantiomer, that is to say no more than about 5% w/w of the (+)- enantiomer, preferably no more than about 2%, in particular less than about 1% w/w is present.

International application PCT/GB91/00706, publication no WO91/17159 describes the preparation of 3TC, its antiviral activity and its use in medicine. 3TC is described and prepared in WO91/17159 as a freeze dried powder.

We have now found that 3TC can be obtained in crystalline form and exhibits polymorphism.

There is thus provided in a first aspect of the invention 3TC in crystalline form.

When crystallised from aqueous solution 3TC is obtained in the form of needle-shaped crystals (hereinafter Form I). In this form the crystals are not favoured for pharmaceutical formulation into solid dosage forms because of their physical properties, for example poor flow characteristics. We have further found that under certain conditions 3TC may be obtained in the form of substantially bipyramidyl crystals (hereinafter Form II). The crystal habit of Form II has improved flow characteristics and is thus preferred in the manufacture of solid dosage forms. In addition Form I crystals are a less stable polymorphic forms and certain pharmaceutical unit operations such as milling may cause conversion of

Form I to Form II, an undesirable characteristic for manufacture of solid dosage forms.

3TC in the form of bipyramidyl crystals has a melting point of greater than about 170^0C, in particular 177-178^0C when pure. 3TC in the form of needle-like crystals has a melting point of less than about 130^0C in particular about 124-127^0C in pure form.

3TC in Form II exhibits characteristic absorption bands in its infra red (i.r.) spectrum which are absent from the i.r. spectrum of Form I. In particular Form II exhibits strong absorption bands at ~920 and ~850 wavenumbers. Further, a characteristic band of Form I at 1110 wavenumbers is absent from the spectrum of Form II.

Form II of 3TC further shows a characteristic endotherm with an onset temperature at 177-178^0C in its differential scanning calorimetry (DSC) profile. By contrast Form I shows a characteristic endotherm in its DSC profile with an onset temperature at 124-127^0C.

There is thus provided in a further aspect of the invention 3TC in the form of needle shaped crystals.

In a further aspect there is provided 3TC in the form of bipyramidal crystals.

In a yet further aspect of the invention there is provided 3TC in crystalline form and having a melting point of greater than 170^0C, in particular 177-178^0C. In an alternative aspect there is provided 3TC in crystalline form and having in its DSC profile an endotherm with an onset temperature of 177-178^0C.

In a yet further alternative there is provided 3TC in crystalline form and having absorption bands at about 920 and about 850 wavenumbers in its infra red spectrum. In particular there is provided 3TC in which in addition to absorption bands at these wavenumbers a band at 1110 wavenumbers is substantially absent.

3TC may be obtained from its racemate by resolution by any method known in the art for the separation of racemates into their constituent enantiomers. In particular 3TC may be obtained from the known racemate by chiral HPLC, by enzyme mediated enantioselective catabolism with a suitable enzyme such as cytidine deaminase or by selective enzymatic degradation of a suitable derivative

using a 5'-nucleotide. Suitable methods for preparing 3TC are described in WO91/17159.

3TC in the form of needle shaped crystals may be obtained by crystallisation of the compound from aqueous solution or by azeotropic distillation with propan-1-ol.

3TC in the form of the preferred bipyramidyl shaped crystals may be obtained by recrystallisation from non-aqueous media, in particular a lower (C_{2-6}) alcohol, for example ethanol, IMS (industrial methylated spirit) or propan-1-ol. In a preferred method 3TC in bipyramidyl form may be obtained from 3TC in needle form by ageing the latter in Industrial Methylated Spirit (IMS) or ethanol at elevated temperature (e.g. 30-70^0, particularly about 50^{0}C) for an appropriate time (e.g. 0.5-3hrs, in particular about 1 hour or more).

Alternatively 3TC in bipyramidyl form may be obtained by heating the compound in needle form above its melting point of 124-127^0, in particular above about 170^{0}C, for example above about 177-178^{0}C and allowing the melt to cool.

In a further alternative 3TC in bipyramidyl form may be obtained by grinding or milling the compound in the form of needle shaped crystals.

Preferably 3TC is in the form of bipyramidyl shaped crystals substantially free of needle crystals. Where these crystals are obtained by recrystallisation or ageing in liquid media the compound will normally be obtained entirely free of needle shaped crystals.

3TC in crystalline form may be used as an antiviral agent as described in WO91/17159 which is incorporated herein by reference.

3TC in crystalline form may be formulated as a pharmaceutical formulation for use as an antiviral agent in described in WO91/17159.

Figure 1 shows 3TC in the form of needle shaped crystals (Form I).

Figure 2 shows 3TC in the form of bipyramidyl shaped crystals (Form II).

Figure 3 is an infra-red spectrum of Form I crystals.

Figure 4 is an infra-red spectrum of Form II crystals.

Figure 5 is a DSC thermogram of Form I crystals.

Figure 6 is a DSC thermogram of Form II crystals.

The following examples illustrate the invention but are not intended as a limitation thereof. All temperatures are in ^0C.

INTERMEDIATE 1

5-Methoxy-1,3-oxathiolane-2-methanol, benzoate.

A solution of zinc chloride (1.6g) in hot methanol (15ml) was added to a stirred solution of mercaptoacetaldehyde, dimethyl acetal (34.2g) and benzoyloxy acetaldehyde (48.3g) in toluene (1300ml) which was then heated to reflux under nitrogen for 50 min. The cooled mixture was concentrated, diluted with some toluene, then filtered through Kiesulguhr. The combined filtrates and toluene were washed with aqueous saturated sodium bicarbonate solution (x2) and brine, dried ($MgSO_4$) then evaporated to an oil which was subjected to column chromatography on silica (2kg, Merck 9385) eluted with chloroform to give the title product as an oil (45.1g) a mixture of anomers (ca 1:1); 1H NMR (DMSO-d_6) 3.1-3.3(4H), 3.42(6H), 4.4-4.6 (4H), 5.41(1H), 5.46 (1H), 5.54 (1H), 5.63 (1H), 7.46 (4H), 7.58 (2H), 8.07 (4H);γmax ($CHBr_3$)1717.6cm^{-1}.

INTERMEDIATE 2

(±)-cis-1-(2-Benzoyloxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-4-dione

A mixture of finely ground uracil(9.62g) hexamethyl disilazane (50 ml) and ammonium sulphate (30 mg) was heated at reflux under nitrogen until a clear solution was obtained. This was cooled and then evaporated to a colourless oil, which was dissolved, under nitrogen atmosphere, in acetonitrile (100ml). The solution was added to a stirred ice cooled solution of 5-methoxy-1,3-oxathiolane-2-meth ol, benzoate (intermediate 1) (19.43g), in acetonitrile (600ml) and trimethyl silyl trifluoromethanesulphonate (14.7ml) was added. The ice bath was removed, and the solution was heated at reflux under nitrogen for 45 mins. After cooling and evaporation, the residue was purified by column chromatography over 1kg of silica gel (Merck 9385) eluting with chloroform/methanol 9:1. Appropriate fractions were cooled and evaporated to afford a crude residue. This was fractionally crystallized from the minimum of hot methanol (c.1200ml) to afford the title compound (6.32g)

as white crystals. 1H NMR(d^6DMSO) δ 11.36 (1H,bs). 7.50-8.00 (6H,m), 6.20 (1H,t), 5.46 (2H,m), 4,62 (2H, m), 3.48 (1H, m), 3.25 (1H, m).

INTERMEDIATE 3

(±)-(cis)-4-Amino-1-(2-benzoyloxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

Method (a)

A suspension of cytosine (20.705g) and ammonium sulphate (few mgs) in hexamethyldisilazane (110ml) was stirred and heated at reflux for 2½h, under nitrogen. Solvent was removed by evaporation, and the residual solid was dissolved in dry acetonitrile (350ml). This solution was transferred using flexible needle techniques into a stirred, ice-chilled solution of 5-methoxy-1,3-oxathiolane-2-methanol, benzoate (Intermediate I) (43.57g) in acetonitrile (650ml) under nitrogen. Trimethylsilyl trifluoromethanesulphonate (33ml) was added, the solution was allowed to warm to ambient temperature (1½h) then heated to reflux for an overnight period. The residue mixture was concentrated, diluted with saturated aqueous sodium bicarbonate solution (500ml), then extracted with ethyl acetate (3x500ml). The combined extracts were washed with water (2x250ml) and brine (250ml) dried ($MgSO_4$) then evaporated to a foam which was subjected to column chromatography on silica (600g, merck 7734), eluted with ethyl acetate-methanol mixtures to give a mixture of anomers (ca 1:1 31.59g). The mixture was crystallised from water (45ml) and ethanol
(9.0ml) to give a solid (10.23g) which was recrystallised from ethanol (120ml) and water (30ml) to give the title product as a white solid (9.26g); λmax (MeOH) 229.4mm ($E^{1\%}_{1cm}$ 610); 272.4mm ($E^{1\%}_{1cm}$ 293); ^{1}H NMR (DMSO d6) δ 3.14 (1H), 3.50 (1H), 4.07 (2H), 5.52 (1H), 5.66 (1H), 6.28 (1H), 7.22 (2H), 7.56 (2H), 7.72 (2H), 8.10 (2H).

Method (b)

Phosphorus oxychloride (7.0ml) was added dropwise to a stirred , ice-cooled suspension of 1,2,4-triazole (11.65g) in acetonitrile (120ml) then, keeping the internal temperature below 15^0C, triethylamine (22.7ml) was added dropwise. After 10 min a solution of (±)-cis -1-(2-benzoyloxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2,4-dione (Intermediate 2) (6.27g) in acetonitrile (330ml)was slowly added. Stirring was then continued at room temperature overnight. The mixture was cooled by means of an ice bath and triethylamine (30ml) was slowly added followed by water (21ml). The resultant solution was evaporated, and the residue was partitioned between saturated sodium bicarbonate solution (400ml) and chloroform (3x200ml). The combined chloroform extracts were dried and magnesium sulphate, filtered and evaporated to give a crude residue (9.7g). The residue was dissolved in 1,4-dioxan (240ml) and concentrated aqueous ammonia solution (s.g 0.880, 50ml) was added. After 1½h the solution was evaporated and the residue dissolved in methanol. This caused precipitation of a solid, which was filtered off. The mother liquors were purified by column chromatography over silica gel (Merck 9385, 600g). Appropriate fractions were pooled and evaporated to give the title compound as a fawn solid (2.18g), identical to that obtained by Method (a).

INTERMEDIATE 4

(±)-(cis)-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

A suspension of (cis)-4-amino-1-(2-benzoyloxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one (Intermediate 3) (8.19g) and Amberlite IRA-400 (OH) resin (8.24g) in methanol (250ml) was stirred and heated to reflux for 1¼h. Solids were removed by filtration then washed with methanol. The combined filtrates were evaporated. The residue was triturated with ethyl acetate (80ml). The resulting white solid was collected by filtration to give the title product (5.09g), 1H NMR (DMSO-d_6) 3.04 (1H), 3.40 (1H), 3.73 (2H), 5.18 (1H), 5.29 (1H), 5.73 (1H), 6.21 (1H), 7.19 (2H), 7,81 (1H).

INTERMEDIATE 5

(-)-cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H) pyrimidin-2-one

(i) Three 50ml flasks of nutrient broth (Oxoid Ltd) were inoculated with a loopful each of Escherichia coli (ATCC 23848) scraped from a Nutrient Agar plate. The flasks were incubated overnight at 37^0C with shaking at 250 rev/min and then each flask was used to inoculate 4l of CDD medium (glutamic acid, 3g/l; $MgSO_4$, 0.2g/1: K_2SO_4, 2.5g/1; NaCl, 2.3g/l, $Na_2HPO_42H_20$, 1.1g/1, $NaH_2PO_42H_20$ 0.6g/l cytidine, 1.2g/l) in a seven litre fermenter. The cultures were fermented at 750 rev/min, 37^0C with aeration at 4l/min. After growth for 24hrs the cells were collected by centrifugation (5000g, 30 minutes) to yield 72g wet weight. The cell pellet was resuspended in 300ml of 20mM Tris HCl buffer (pH 7.5) and disrupted by sonication (4 x 45 seconds). The cell debris was removed by centrifugation (30,000 g, 30 minutes) and the protein in the supernatant was precipitated by addition of ammonium sulphate to 75% saturation. The precipitate was collected by centrifugation (30,000g. 30 minutes) and the pellet was resuspended in 25ml of HEPES buffer (100mM, pH 7.0) containing ammonium sulphate (75% saturation). Enzyme solution was prepared by centrifugation at 12,000 rpm for 30 mins. The supernatant was discarded and the pellet dissolved in Tris HCl buffer (pH 7.0; 100mM) to the original volume.

(ii) Intermediate 4 (115mg was dissolved in water (100ml), and stirred. Enzyme solution (0.5ml) was added, and the mixture was maintained at a constant pH by the continual addition of HCl (25mM). The conversion was monitored by chiral HPLC, which showed that the (+) enantiomer of the substrate was preferentially deaminated. After 22hr the (+) enantiomer of the substrate (RT 12.5min) had been completely removed, and the solution was adjusted to pH 10.5 by the addition of conc. sodium hydroxide.

The solution produced above was eluted through a column of QAE Sephadex (A25; Pharmacia; 30X 1.6cm), pre-equilibrated to pH11. The column was washed with water (200ml) and then with HCl (0.1M). Fractions (40ml) were taken , and

analysed by reversed phase HPLC. Fractions 5-13, containing the unreacted (-) enantiomer of the substrate, were combined and adjusted to pH 7.5 with HCl. Fraction 47, containing deaminated product, was adjusted to pH7.5 with dil. NaOH. Analysis by chiral HPLC showed that this material was a mixture, consisting of one enantiomer (RT 10.2min) as the major component with the other enantiomer (RT 8.5min) as a minor component (e.e ca 90%).

(iii) Stage (ii) above was repeated on a larger scale . The compound of Example 1 (363mg) in 250ml of water was incubated with enzyme solution (0.5ml), prepared as in Stage (i). Further aliquots (0.5ml) of enzyme were added after 18 and 47 hrs. The reaction mixture was stirred for 70hr., then left standing for a further 64hr. Analysis by chiral hplc indicated that the (+) enantiomer of the substrate had been completely deaminated, and the resulting solution was adjusted to pH10.5 with NaOH.

The solution above was loaded onto the same QAE column, and eluted as in stage (i). Fractions 2-6, containing a mixture of the residual substrate and deaminated product, were bulked. Fractions 7-13, containing the residual substrate ((-) enantiomer), were bulked and adjusted to pH7.5. Fractions 25-26, containing deaminated product, were bulked and neutralised

Fractions 2-6 above were re-eluted through the same QAE column. Fractions 3-11 from this second column contained unrected substrate ((-) enantiomer). Fraction 70 contained the deaminated product.

(iv) The resolved substrate fractions from stage (ii) and (iii) were combined and adjusted to pH7.5. This solution was eluted through a column of XAD-16 (40x2.4cm), packed in water. The column was washed with water, and then eluted with acetone: water (1:4 v/v). Fractions containing the desired (-) enantiomer were bulked and freeze-dried to give a white powder (190mg).

The HPLC methods used above were as follows:-

1. Reversed Phase analytical HPLC

Column	:	Capital Cartridge Spherisorb ODS-2 (5uM) 150x4.6mm
Eluant	:	Ammonium dihydrogen phosphate (50mM)+ 5% MeCN
Flow	:	1.5ml/min
Detection	:	UV, 270nm
Retention Times	:	BCH 189 5.5min
	:	deaminated BCH -189 8.1min

2. Chiral analytical HPLC

Column	:	Cyclobond I Acetyl 250x4.6mm
Eluant	:	0.2% Triethylammonium acetate (pH7.2)
Flow	:	1.0ml/min
Detection	:	UV, 270nm
Retention Times	:	BCH 189 11.0 and 12.5min
	:	deaminated BCH-189 8.5 and 10.2 min (The bioconversion was followed by monitoring the loss of the peak at 12.5min., and accumulated of product at 10.2min).

Example 1

A suspension of Intermediate 5 (64.8g) in water (200mL) was heated to 45^0 to give a solution. The solution was cooled to 30^0.

The product crystallised as an unstirrable mass. This was broken up and the suspension stirred at ca. 10^0 for 1h.

The product was isolated by filtration and washed with ethanol (IMS; 2 x 30mL) then dried in vacuo at 45^0 for 24h to give 3TC as Form I (fine needle crystals).

The compound had an i.r. spectrum and DSC thermograph identical to Figures 3 and 5 respectively.

Example 2

A suspension of the compound of Example 1 (10.0g) in industrial methylated spirits (IMS; 200mL; 20 volumes) was heated to reflux to give a clear solution. The solution was filtered hot and the filtrate was distilled at atmospheric pressure until 100mL (10 volumes) of solution remained. The solution was seeded with authentic material[2] and allowed to cool from 80^0 to 25^0 over 1h. Crystallisation began at 79^0. The suspension was stirred at 15^0 for 1h. The product was isolated by filtration and washed with IMS (10mL; 1 volume). Drying in vacuo at 50^0 gave the title compound as aggregates of bipyramides (8.42g) m.p. 179-181^0. (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one.

Assay Found : C,41.9; H,4.85; N,18.35

$C_8H_{11}N_3O_3S$ requires : C,41.9; H,4.8; N,18.3%

The compound had an i.r. spectrum and DSC thermograph identical to Figures 4 and 6 respectively.

Example 3

A suspension of the product of Example 1 (20.0g) in Industrial Methylated Spirits (IMS; 100mL; 5 volumes) was stirred slowly at 50^0 for 1h.

A small sample (ca 100mg) was removed, dried in vacuo at 50^0 and examined by microscopy and differential scanning calorimetry (DSC).

The sample was 100% Form II (bipyramidal habit).

The suspension was stirred at 50^0 for a further 2h and a sample removed. Microscopy showed no change.

The suspension was stirred at 50^0 for 22h, then cooled to 20^0 and stirred for 1h.

The suspension was filtered, the product washed with IMS (20mL; 1vol) and dried in vacuo to give as a white crystalline solid (17.13g) (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyramidin-2-one m.p. 180-181^0.

Assay Found : C,41.85; H,4.85; N,18.3

$C_8H_{11}N_3O_3S$ Requires : C,41.9; H,4.8; N,18.3%

The product had an i.r. spectrum and DSC thermogram identical to those of Figures 4 and 6 respectively.

Example 4

X-Ray Crystallography data for Form II

Crystal Data :

$C_8H_{11}N_3O_3S$, M = 229.26.

Tetragonal, a = b = 8.749(3), c = 26.523(9)A, V= 2030(2)A^3

(by least-squares refinement on diffractometer angles for 14 automatically centred reflections, 1 = 1.54184A).

Space group $P4_32_12$ (No. 96), z = 8, D_c = 1.50g cm^{-3}.

F(000) = 960, m(Cu-Ka) = 27.5 cm^{-1}.

Dimensions of data crystal 0.48 x 0.32 x 0.30 mm.

Single crystals of Form II (colourless bipyramids) were examined by X-ray diffraction. A total of 1651 reflections were measured ($3 < 2J < 115^0$) on a Siemens R^3m/V diffractometer with monochromatised Cu-Ka radiation and using 2J/w scans. The structure was solved by direct methods and the non-hydrogen atoms refined anisotropically. The hydrogen atoms attached to carbon were idealised (C-H = 0.96A) and allowed to ride on their parent carbon atoms. Three Hs on $-NH_2$ and -OH groups were located from a difference Fourier map. All H atoms were refined isotropically. Refinement converged to give R = 0.068, R_w = 0.069, $w^{-1} = [s^2(F) + 0.005[F]^2]$. Maximum residual electron density was 0.45 eA^{-3}. The absolute chirality was confirmed using Rogers' eta test [h = 0.99 (9)].

Example 5

Pharmaceutical Formulations

(a) 100mg Tablets

Ingredients per tablet	
3TC (Form II)	100.0mg
Microcrystalline Cellulose NF	189.5mg
Sodium Starch Glycolate NF	9.0mg
Magnesium Stearate NF	1.5mg
Total Weight	300.0mg

The 3TC (Form II), microcrystalline cellulose and sodium starch glycolate were sieved and blended in a V-blender for about 15 minutes. Sieved magnesium stearate was then added and blending continued for a further 2 minutes.

The blend was compressed in standard tabletting equipment and then film coated with an aqueous suspension of grey Opadry to produce aesthetically acceptable tablets.

(b) 300mg Tablets

Ingredients per tablet	
3TC (Form II)	300.0mg
Microcrystalline Cellulose NF	279.0mg
Sodium Starch Glycolate NF	18.0mg
Magnesium Stearate NF	1.5mg
Total Weight	600.0mg

Tablets were prepared as described in (a) above.

CLAIMS

1. (-)cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in crystalline form.
2. (-)-cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin -2-one in the form of bipyramidyl crystals.
3. The crystalline form as claimed in claim 1 or claim 2 having a melting point of greater than 170°C.
4. The crystalline form as claimed in any one of claims 1 to 3, having a melting point of 177-178°C.
5. The crystalline form as claimed in any one of claims 1 to 4 having absorption bands in its infra-red spectrum of 920 and 850 wave numbers.
6. The crystalline form as claimed in any one of claims 1 to 5 having no absorption band in its infra-red spectrum at 1110 wave numbers.
7. The crystalline form as claimed in any one of claims 1 to 6 having an endotherm with an onset temperature at 177-178°C in its differential scanning calorimetry profile.
8. (-)cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of needle shaped crystals.
9. The crystalline form as claimed in claim 1 or claim 8 having a melting point of less than about 130°C.
10. The crystalline form as claimed in claim 1, claim 8 or claim 9 having a melting point of 124-127°C.
11. The crystalline form as claimed in claim 1, or any one of claims 8-11 having an endotherm with an onset temperature of 124-127°C in its differential scanning calorimetry profile.
12. The crystalline form as claimed in claim 1 or any one of claims 8 to 11 having an absorption band in its infra-red spectrum at about 1110 wave numbers.
13. (-)cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the crystalline form as shown in Figure 1.
14. (-)cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the crystalline form as shown in Figure 2.

15. (-)cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in crystalline form and having an infra-red spectrum as shown in Figure 3.

16. (-)cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in crystalline form and having an infra-red spectrum as shown in Figure 4.

17. A method for the preparation of (-)-cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in crystalline form which comprises crystallisation of the compound from aqueous solution.

18. A method for the preparation of (-)-cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in crystalline form which comprises areotropic distillation of an aqueous solution of the compound with propan-1-ol.

19. A method for the preparation of (-)-cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in crystalline form which comprises recrystallisation from non-aqueous medium.

20. A method as claimed in claim 19 wherein the non-aqueous medium is a C_{2-6} alcohol.

21. A method as claimed in either claim 20 or claim 21 wherein the non-aqueous medium is selected from ethanol and industrial methylated spirit.

22. A method for the preparation of (-)-cis-4-Amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of bypyramidyl crystals which comprises aging the compound in the form of needle shaped crystals in ethanol or industrial methylated spirits at elevated temperature.

23. A pharmaceutical formulation comprising (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in crystalline form and a pharmaceutically acceptable carrier therefor.

24. A pharmaceutical formulation as claimed in claim 23 in a form suitable for oral administration.

25. A pharmaceutical formulation as claimed in claim 23 or claim 24 in the form of a tablet or capsule.

FIG.1

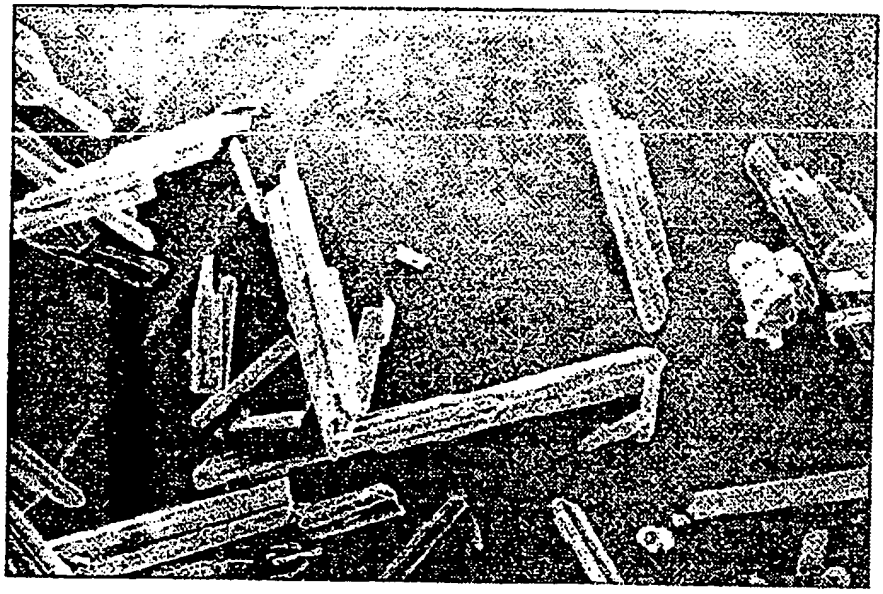


FIG.2

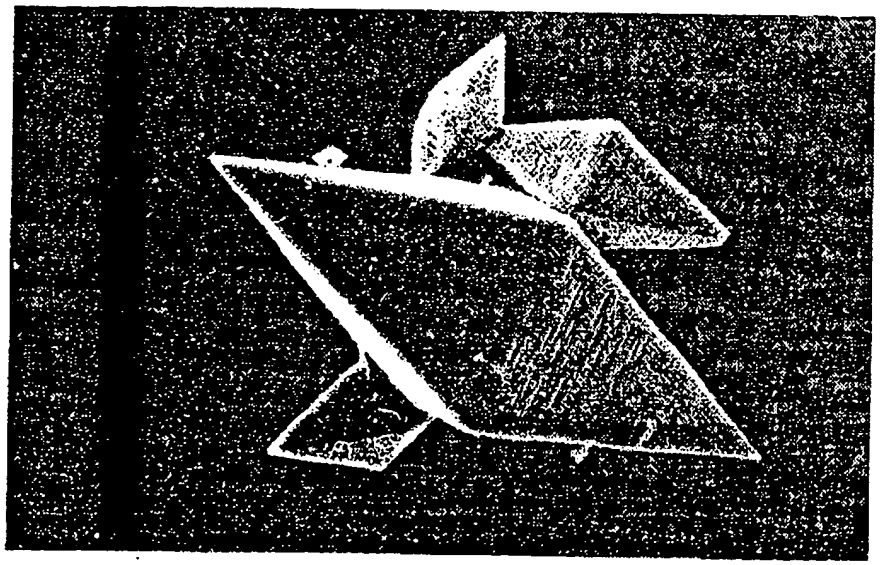


FIG.3

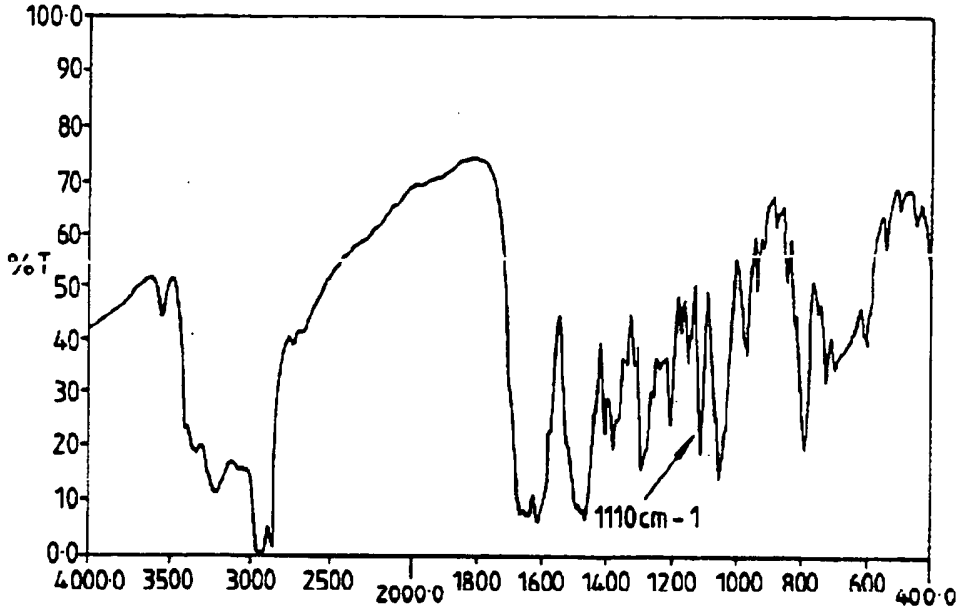

100·0
90
80
70
60
%T
50
40
30
20
10
0·0
4000·0 3500 3000 2500 2000·0 1800 1600 1400 1200 1000 800 600 400·0
1110cm-1

FIG.4

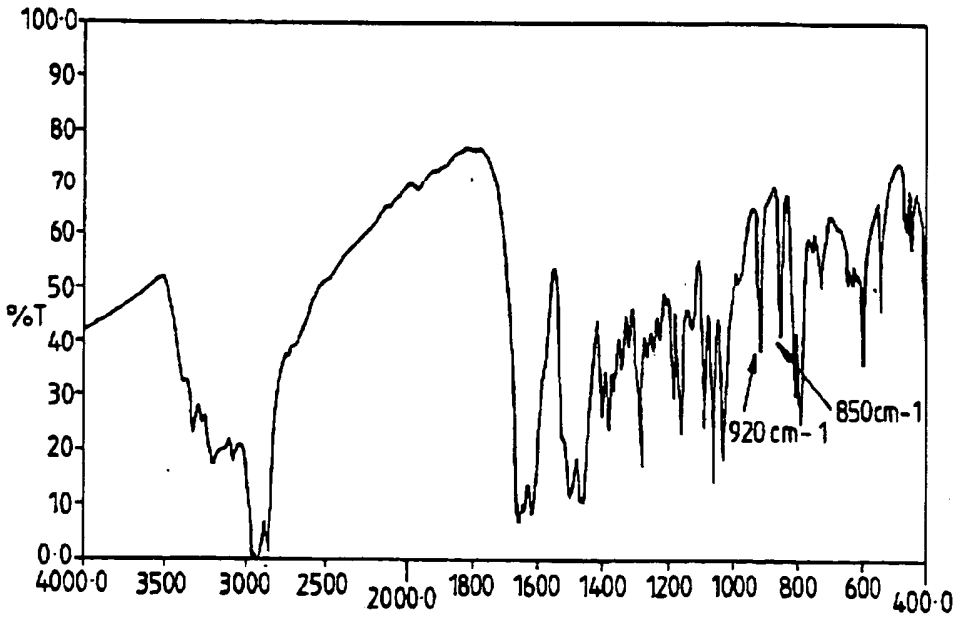
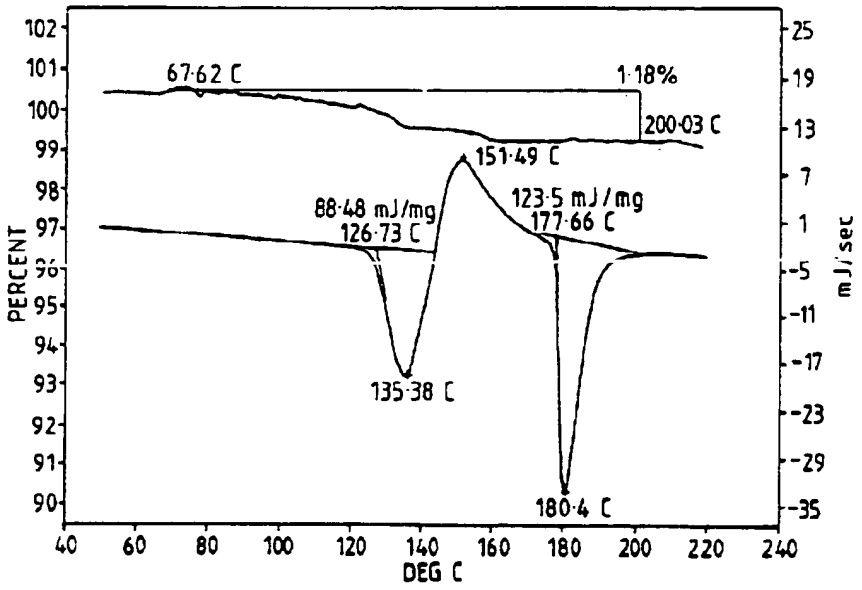

100·0
90
80
70
60
%T
50
40
30
20
10
0·0
4000·0 3500 3000 2500 2000·0 1800 1600 1400 1200 1000 800 600 400·0
920 cm-1
850cm-1

FIG.5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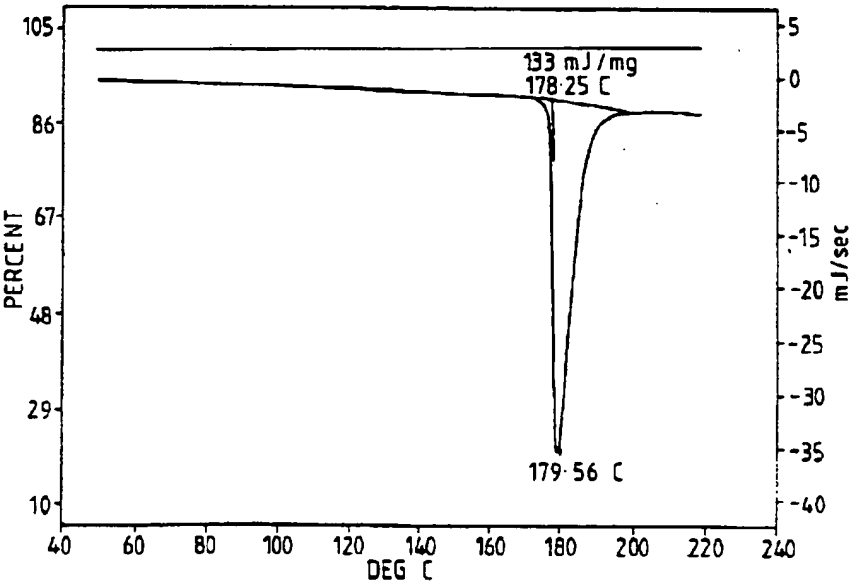
1·18%
200·03 C
151·49 C
88·48 mJ/mg
126·73 C
123·5 mJ/mg
177·66 C
135·38 C
180·4 C
PERCENT
mJ/sec
DEG C

FIG.6


133 mJ/mg
178·25 C
179·56 C
PERCENT
mJ/sec
DEG C

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/01213

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D411/04; A61K31/505

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 382 526 (IAF BIOCHEM INTERNATIONAL) 16 August 1990 cited in the application see claims; example 7	1,23-25
A	CHEMICAL ABSTRACTS, vol. 115, 1991, Columbus, Ohio, US; abstract no. 45942T, R. ROOKE ET AL.: 'BIOLOGICAL COMPARISON OF WILD TYPE AND ZIDOVUDINE-RESISTANT ISOLATES OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 FROM THE SAME SUBJECT' page 486 ;column 1 ; see abstract & ANTIMICROB. AGENTS CHEMOTHER. vol. 35, no. 5, 1991, MONTREAL pages 988 - 991;	1,23-25

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15 SEPTEMBER 1992	07. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	FRANCOIS J.C.

Form PCT/ISA/210 (second sheet) (January 1985)